

Attorney Docket No.: UT-0006
Inventors: Rao et al.
Serial No.: 09/109,858
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12. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;

(b) incubating the multipotent CNS stem cells in NEP medium;

(c) replating the multipotent CNS stem cells on laminin in NEP medium in the absence of chick embryo extract to induce cell differentiation;

(d) purifying from the differentiating cells a subpopulation of cells expressing embryonic neural cell adhesion molecules via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, wherein said procedure uses an embryonic neural cell adhesion molecule antibody; and

(e) incubating the purified subpopulation of cells in a FGF-containing medium configured for supporting adherent growth thereof to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells, wherein said neuron-restricted precursor cells differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic

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acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

21. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube but prior to differentiation of glial and neuronal cells in the neural tube;

(b) dissociating cells comprising the sample of spinal cord tissue removed from the embryo;

(c) purifying from the dissociated cells via an embryonic neural cell adhesion molecule antibody a subpopulation expressing embryonic neural cell adhesion molecule;

(d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(e) incubating the plated cells at a temperature and in an atmosphere conducive to growth to obtain an isolated, pure population of neuron-restricted precursor cells, wherein said

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neuron-restricted precursor cells require FGF for adherent growth, differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

28. (amended) A method of producing rodent or human postmitotic neurons from rodent or human neuron-restricted precursor cells comprising:

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(a) culturing rodent or human neuron-restricted precursor cells which require FGF and differentiate into CNS neuronal cells but not into CNS glial cells in proliferating conditions; and

(b) changing the culture conditions of the rodent or human neuron-restricted precursor cells from proliferating conditions to differentiating conditions, thereby causing the neuron-restricted precursor cells to differentiate into postmitotic neurons.

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59. (amended) A method of isolating a pure population of mouse or human CNS neuron-restricted precursor cells comprising the steps of:

(a) providing a sample of mouse or human embryonic stem cells;

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4 (b) purifying from the mouse or human embryonic stem cells via an embryonic neural cell adhesion molecule antibody a subpopulation expressing embryonic neural cell adhesion molecule;

(c) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(d) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.

REMARKS

Claims 12, 15, 16, 21, 23, 24, 26-33 and 59 are pending in the instant application. Claims 12, 15, 16, 21, 23, 26-33 and 59 have been rejected. Claims 12, 21, 28 and 59 have been amended. No new matter has been added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.